High Pressure Processing of Corn and Wheat Starch

A Thesis

Presented in fulfillment of the Requirements for Graduation with Distinction in the Department of Food, Agricultural, and Environmental Engineering at

The Ohio State University

By

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Abstract

High hydrostatic pressure (HHP) has been investigated for food preservation as an alternative to thermal processing. HHP has been known to affect high molecular weight polymers causing phase change. Starch has been gelatinized on the order of 600-700MPa, at 25°C. Gelatinized starch recrystallizes during storage affecting the texture and shelf life of food products. HHP was applied to corn and wheat starch to study the effects of storage temperature and time on recrystallization of starch in HHP induced starch gels and on the stability of aqueous suspensions of starch.

The effect of storage temperature on re-crystallization kinetics of gelatinized corn and wheat starch were evaluated using a differential scanning calorimeter. The textural properties of gelatinized corn and wheat starches during storage were monitored as a function of time using a rheometer. The rate of retrogradation depended on the storage temperatures (23°C and 4°C) and the botanical origin of starch. The corn and wheat starch stored at 23°C exhibited characteristics of strong gels for a longer period of time than that of the starches stored at 4°C.

Pregelatinizing starch is a method used to modify native starch in order to improve several characteristics including increased solubility. Currently pregelatinized starches are prepared by thermal processing. Thermal and HHP processed pregelatinized corn and wheat starch were compared to native corn and wheat starch to determine the settling characteristics of the starches. The settling characteristics were determined by measuring the turbidity of the corn and wheat starch as a function of time with a spectrophotometer, and by measuring the amount of solid separation and suspension after 24 hours of settling. The data collected show that HHP and thermal processing have advantageous effects for industrial use compared to native corn and wheat starch. The results also show that the settling characteristics depend on the processing conditions and biological origin of the starch.

Acknowledgments

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I would also like to thank the United States Department of Agriculture and the Ohio State Engineering Honors Department for funding my research; without their monetary support this project would not have been possible. Special consideration is given to Eric Plum, who graciously offered the use of his spectrophotometer to collect data for the transmittance section of my research project. Without his generosity and kindness, this project would not have been as successful. I would also like to thank Kurt Koelling, who gave his time to be on my thesis committee.

I am also grateful to my fiancée, Ellen, who encouraged me to take on the task of writing an honors thesis. She has consistently supported me throughout my research and has always firmly believed in my abilities. Her support has helped me to stay optimistic and goal-oriented.

Last, but not least, I would like to thank my family. My parents, John and Sandy, for their unconditional love and support, for teaching me to value my work, for the

encouragement to always follow my interests and chase my dreams, and for always believing in me. My sister, Ann Marie, who has listened to my frustrations, given me support, taught me many important life lessons, and has graciously permitted me to reside in her home throughout my college career. My brother-in-law, Tim, who has taught me to be patient and to take time for myself when I am stressed. My grandmother, Marcia, who has always freely given her time to me and cared for me throughout my life.

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Introduction

Starches are utilized in many food products to increase viscosity or to form gels. Because starch is insoluble in water, a mixture of starch with water forms a suspension. Starch granules in suspension swell with heat and the viscosity of the suspension increases depending on starch concentration. Thermal processing changes the physico-chemical properties of starch; such, as increased water solubility and viscoelastic behavior (Fennema 1996; Rao 1999; Jobling, 2004). Starches from different biological origins show different physical and chemical properties including gelatinization temperature (52-66ºC for wheat and 62-67ºC for corn) and viscosity after heat treatment (Roos, 1995).

High hydrostatic pressure (HHP) has been shown to affect high molecular weight polymers causing denaturation of proteins (Messens et al, 1997; Famelart et al, 1998) and gelatinization of starch (Douzals et al 1996; Douzals et al, 1998; Chungcheng et al, 1999). Douzals et al. (1998) found that wheat starch granules swelled as a result of HHP treatment. Studies on 16% (w/w) wheat starch showed that starch gelatinization by pressure as evaluated by differential scanning calorimetry (DSC) starts at 300MPa and starch is completely gelatinized at 600-700MPa, at 25˚C (Douzals et al, 1996; Douzals et al, 1998; Zuo, Chuncheng and Chenglin, 1999; Rubens and Heremans, 2000).

Gelatinized starch recrystallizes during storage, affecting the texture and shelf life of food products. This phenomenon is known as retrogradation. Retrogradation contributes to the quality defects in foods such as loss of viscosity and precipitation in soups and sauces. Jouppila et al (1998) reported that water content, storage temperature, and the temperature difference between storage temperature and glass transition temperature were important factors in retrogradation of thermally treated corn starch. The Avrami equation was shown to describe the starch crystallization behavior based on x-ray diffraction (Jouppila et al, 1998) and DSC data (Jouppila and Roos, 1997). Douzals et al (1998) reported that recrystallization of the HHP gelatinized wheat starch (30% dry matter) during storage reached to an asymptotic value after 6 days, and the extent of retrogradation was higher for starch gelatinized by heat than starch gelatinized by pressure at 600MPa. However, Stolt et al (2001) reported that the retrogradation of heat (90ºC, 30 minutes) and pressure (550 MPa, 30ºC, 10 minutes) treated 25% (w/w) barley starch stored at 4˚C were similar and did not reach an asymptotic value after 7 days of storage. Retrogradation depends on the botanical source of the starch, temperature, and starch concentrations. It is important to explore the impact HHP processing on retrogradation characteristics of starches from different botanical origin.

Native starches produce weak bodied, cohesive, rubbery pastes when cooked and undesirable gels when the pastes are cooled. It has been known that starch, pasted and dried without excessive retrogradation, can be re-dissolved in cold water. Such starches, called pregelatinized starch or instant starch, are precooked starches and they are prepared commercially either using drum dryers or extruders. Pregelatinization is one of the methods used to modify the native starch in order to improve several characteristics including increased solubility (Fennema 1996; Walter 1998). It is important to characterize the potential of HHP processing to modify the functional properties of starch.

For successful application of high pressure processed starch to produce commercial food products, it is important to study the impact of HHP on modification of physical properties of starch relevant to food processing and storage. Having knowledge about the physical properties of HHP treated starch is essential to optimize the processing protocols so as to improve the physical stability and textural characteristics of food products.

The purpose of this study is to evaluate the effect of HHP processing on the crystallization kinetics of starch and its effect on rheological characteristics during storage. The stability of aqueous suspensions of HHP processed and dried starch were also explored in terms of settling properties and compared to native starches as well as pregelatinized starches prepared by heat treatment.

Materials and Methods

Crystallization Kinetics of HHP processed starch

Materials

Wheat starch (Sigma, St. Louis, MO) and corn starch (Sigma, St. Louis, MO) were used to prepare starch suspensions. Corn or wheat starch was suspended in distilled water at a concentration of 15% (w/w) starch. Bakeshure 250 (Bachem, Slate Hill, NY), which includes sorbic acid was added at a concentration of 0.1% to the starch suspensions to eliminate mold growth during storage.

High pressure processing of starch suspensions

50mL of the starch suspension was placed inside a sterile polyethylene bag (Fisher Scientific, Canada) and vacuum sealed. The bags were then placed in a hydrostatic high pressure unit (Quintas AFP-6, ABB Autoclave Systems, Columbus, OH). The pressure transmitting medium fluid in the pressure unit contained 50% propylene glycol (Houghton-Safe 620-TY, Houghton Int., Valley Forge, PA) and 50% distilled water. The high pressure processing was performed at 600MPa and 25˚C for 15 minutes.

DSC study

The degree of gelatinization by pressure and crystallization of HHP treated starch as a function of storage time were determined using DSC (model 2090, TA Instruments, New Castle, DE). 50-55 mg samples of HHP treated starch were placed in a high volume stainless steel crucible and the thermograms were recorded from 1°C to 100° at a heating rate of 5°C/min. Each thermogram was analyzed to calculate the onset and the peak temperatures and the enthalpy of the endothermic transition corresponding to the melting of crystallized starch.

Analysis of DSC data

The enthalpy data as a function of time were used to evaluate the crystallization kinetics of the HHP treated starch. Fractional crystallization, α, was defined in terms of the apparent enthalpy of crystallization,

$$\frac{\Delta H_t - \Delta H_i}{\Delta H_f - \Delta H_i} = \alpha \qquad (1)$$

where ΔH_i is the enthalpy measured immediately following the pressure treatment, ΔH_f, and ΔH_t are the enthalpy of melting for the recrystallized starch after crystallization to maximum extent and after time t, respectively. The enthalpy data were fitted to the Avrami equation (Avrami, 1939) to determine crystallization kinetic parameters of HHP treated starch,

$$\frac{\Delta H_t - \Delta H_i}{\Delta H_f - \Delta H_i} = (1 - e^{-kt^n}) \qquad (2)$$

where k and n are constants.

The half time ($t_{1/2}$) for crystallization of the corn and wheat starch was also calculated using the following equation.

$$t_{1/2} = \left(- \frac{\ln(0.5)}{k} \right)^{\frac{1}{n}} \qquad (3)$$

Rheological properties

The rheological properties of the pressure treated corn and wheat starch were studied as a function of storage temperature (23ºC and 4ºC) and time by using a controlled stress rheometer (AR 1000-N, TA Instruments, DE). A 6 cm acrylic cone geometry was used to perform dynamic rheological tests of the pressure treated starch. Stress sweep experiments were used to determine the linear viscoelastic region. Then, frequency sweep tests were performed over a frequency range of 0.1-50Hz to determine the storage modulus (G'), and loss modulus (G").

Analysis of rheology data

The rheological properties were evaluated as a function of time to find the differences in the rheological properties of HHP treated corn and wheat starch. Plots of storage modulus, G' (Pa) at 1Hz versus time, and the slope of loss modulus G" (Pa) versus frequency as a function of time were used to evaluate the textural characteristics of pressure treated starch systems as a function of storage time and temperature.

Analysis of settling characteristics of HHP processed starch in aqueous solution

Materials

Wheat starch (Sigma, St. Louis, MO) and corn starch (PFP, Staley, Decatur, IL) were used to prepare starch suspensions. Starch is exposed to HHP or thermal processing in order to reduce the sedimentation in aqueous suspension.

Processing steps for modification of starch

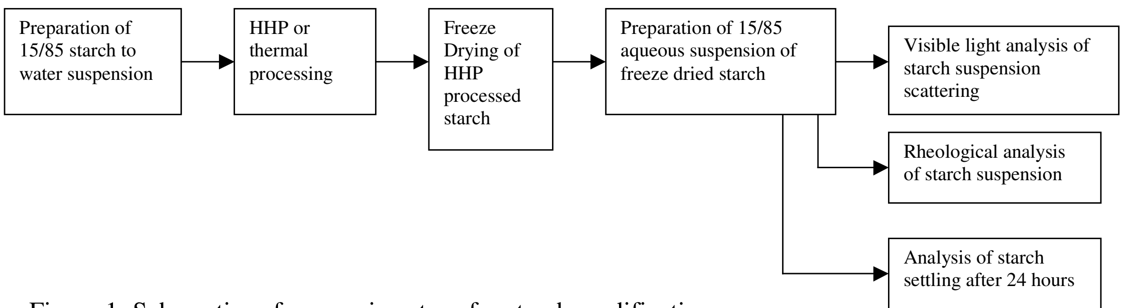

Figure 1: Schematics of processing steps for starch modification.

Starch suspension prior to HHP or thermal processing and after freeze drying was prepared using a buffer system. pH's of buffers used for pre-HHP or thermal processing were 3, 6, and 9 and those of post-freeze drying were 3, 5, and 7. Pressure processing was applied at 25°C and 600MPa for 15 minutes.

Thermal processing was applied to 30mL of corn or wheat starch suspensions at a concentration of 15% starch (w/w). A 50mL centrifuge tube was attached to a ring stand and lowered into boiling water, and was kept in the boiling water for 15 minutes. The starch pastes prepared by HHP or thermal processing were frozen at -80°C for 24 hours and were freeze dried.

Turbidity Measurements

The settling kinetics of corn and wheat starch was studied as a function of processing, pH, and time by using the S2000 miniature fiber optic spectrophotometer (Ocean Optics, Dunedin, FL). The freeze dried starch was suspended in distilled water or in buffer at 5% (w/w) concentrations in 1.2cm x 1.2cm x 4.5cm cuvettes. Visible light spectroscopy was used to monitor the turbidity of starch suspension as a function of time by recording the transmittance of light through the starch suspension. The transmittance readings were collected at wavelengths ranging from 350-750nm as a function of time. Transmittance is the ratio of light transmitted through a solution to the total incident light that could pass through the solution and is expressed as a percent.

The photograph of the setup that was used to record the data for turbidity measurements is shown in figure 2. The set up consists of a power supply, light source, sample chamber, spectrophotometer, and computer (figure 3a). The light is carried in a 5mm fiber optic cable to the sample chamber (figure 3b). The light was passed through the sample at 2.05cm below the sample surface (figure 3b). The light passes through a collimating lens and the collimated light is then sent through the sample. The light transmitted through the sample is focused by a lens on the end of the second optical fiber which goes to the spectrophotometer. Upon entering the spectrophotometer, the light is collimated by a spherical mirror (mirror A) and is diffracted by a plane grating (figure 3c). A second spherical mirror (mirror B) focuses the light so that an image of the spectrum is projected onto a one dimensional charge coupled device (CCD). The CCD converts the light intensity an analog signal and the data are transferred to a computer through an A/D converter. The transmittance data as a function of time were extracted at

600nm to determine and compare the effect of botanical origin and processing on characteristics of starch.

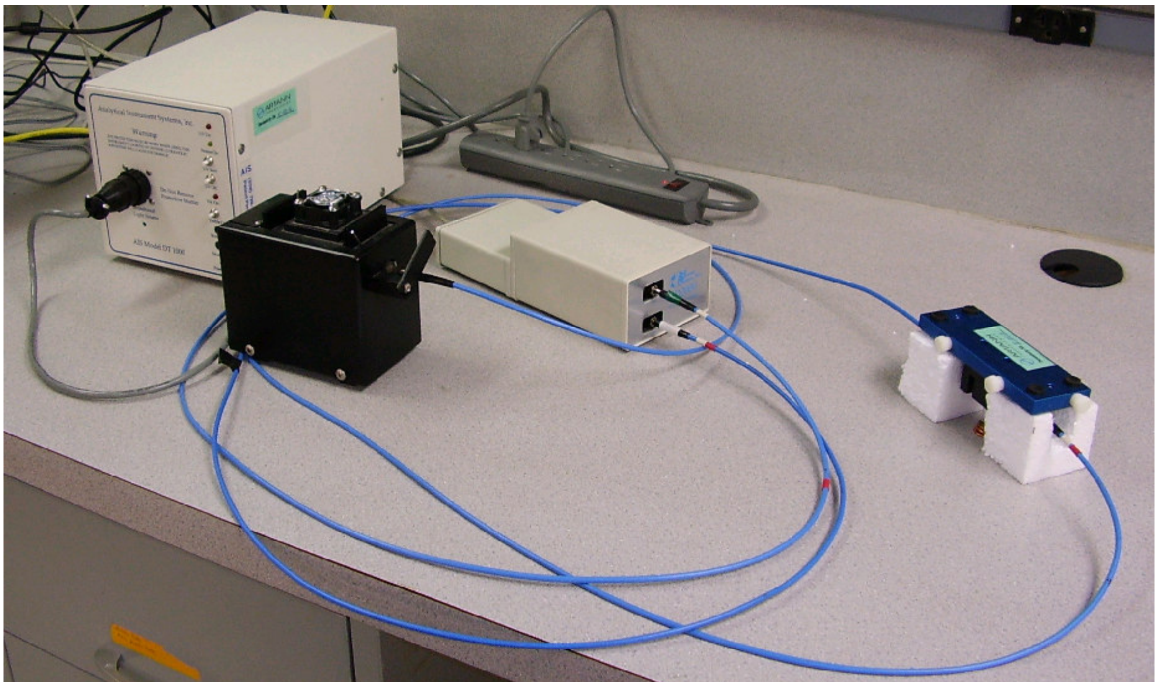

Figure 2: Picture of spectrophotometer setup.

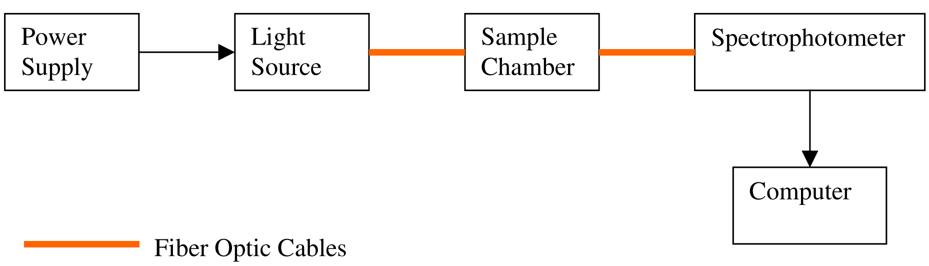

Figure 3a: Schematic of spectrophotometer system.

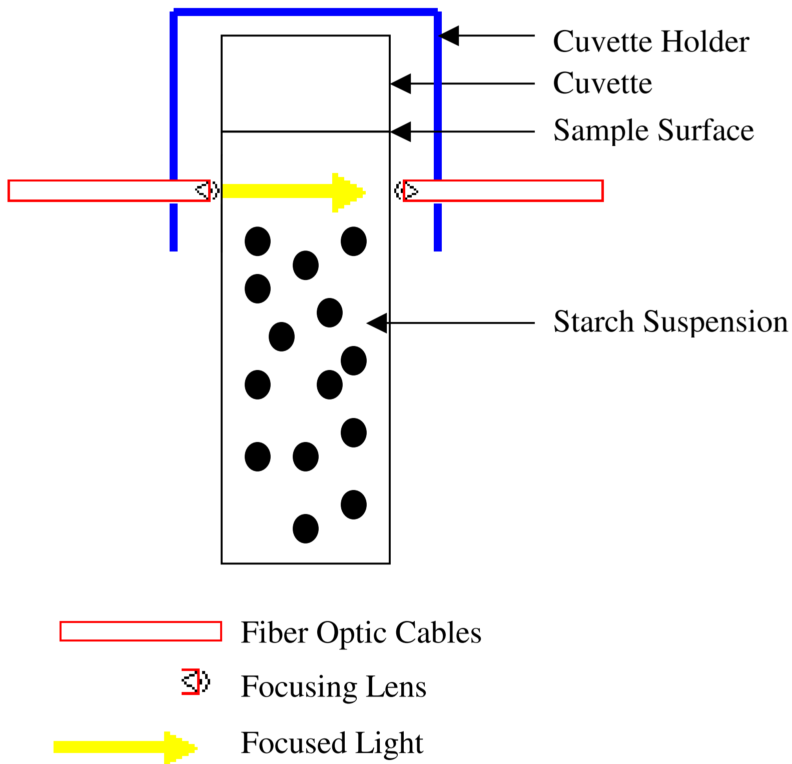

Figure 3b: Detailed drawing of sample compartment and fiber optic cable assembly.

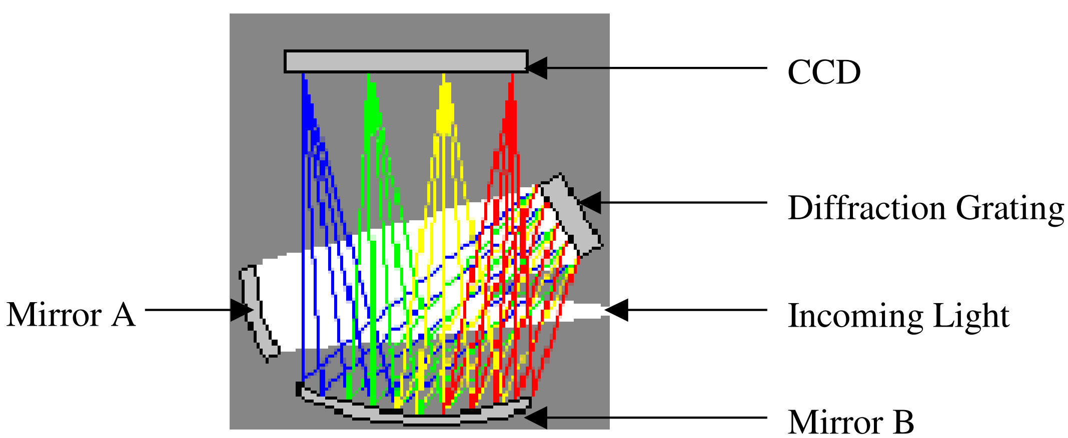

Figure 3c: Detailed drawing of inside of the spectrophotometer.

Phase separation characterization

The phase separation characteristics of HHP and thermally processed corn or wheat starch were studied by preparing a 5% starch solution and measuring the height of settled solid separation in a cuvette. The suspension was placed inside a 1.2cm x 1.2cm x 4.5cm cuvette, and the cuvettes were kept inside an incubator at 25°C. After 24 hours, the heights of settled solid and suspension were measured. The separation data were evaluated by plotting the ratio of suspension and settled solid heights to total height of the initial starch suspension.

Results

The effect of HHP treatment on the starch crystallization during storage and on stability of aqueous starch suspensions were determined. Crystallization was characterized using a DSC and settling characteristics of modified or native starch suspensions were evaluated by monitoring transmittance of light using a spectrophotometer.

Effect of pressure treatment on gelatinization of starch

DSC thermograms of 15% (w/w) corn and wheat starch suspensions show that the two starches have different thermal gelatinization characteristics (Figure 4a).

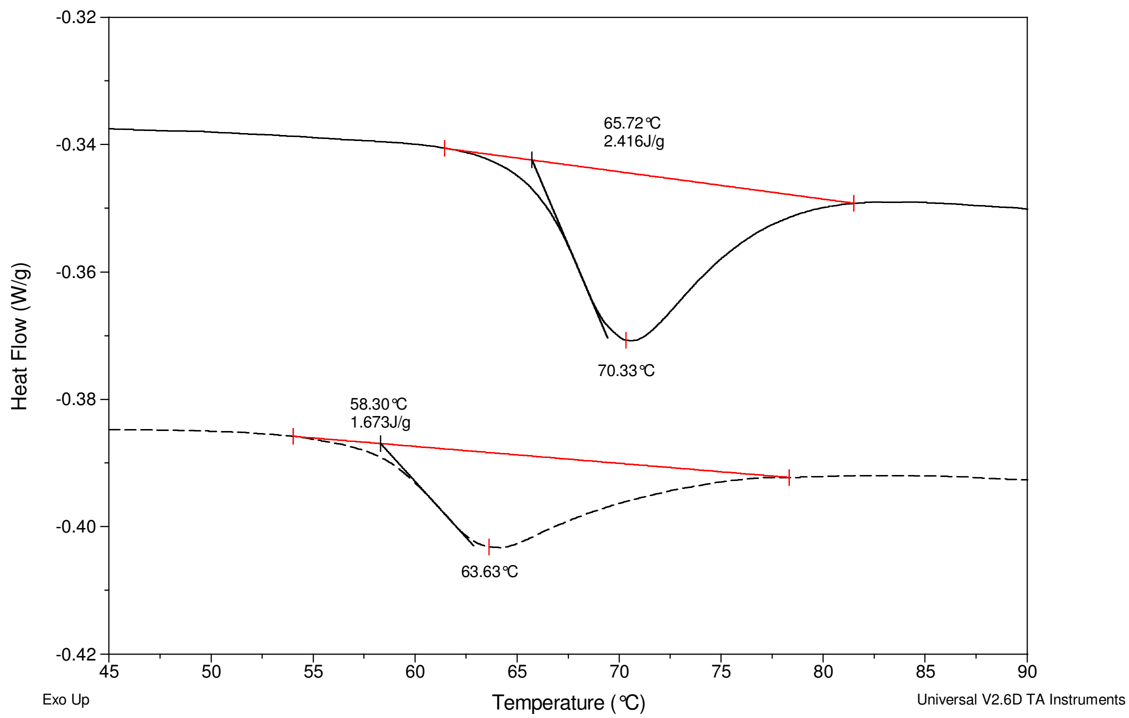

Figure 4a: 15% (w/w) corn and wheat starch suspensions with no pressure treatment. Corn starch (——) and Wheat Starch (------).

Gelatinization temperature of corn starch is higher than that of wheat starch as evaluated in terms of onset (65.7ºC vs 58.3ºC) and peak temperatures (70.3ºC vs 63.6ºC) of gelatinization endotherm. In addition, corn starch required a larger heat energy for gelatinization per gram of dry starch (11.153 Jgds^{-1} vs 16.107 Jgds^{-1}).

HHP treated 15% (w/w) starch water paste did not display any endotherm indicating that both starches were completely gelatinized after a pressure treatment at 600MPa and 25°C for 15 minutes (Figure 4b).

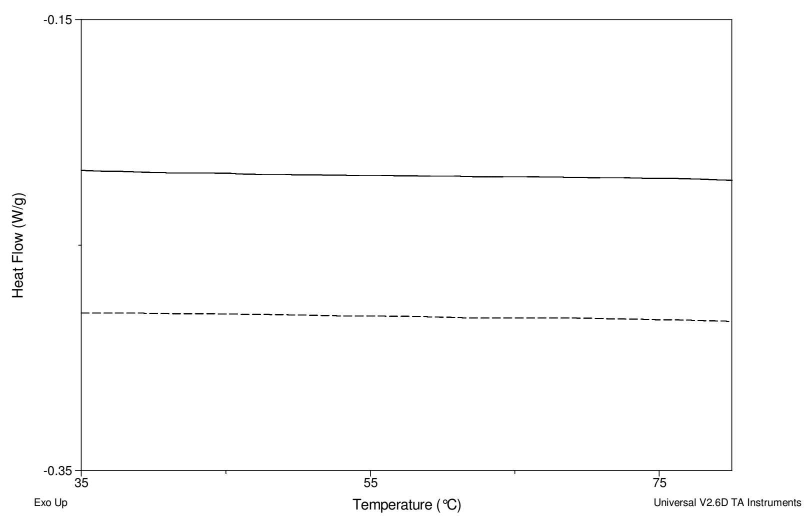

Figure 4b: 15% (w/w) corn and wheat starch suspensions immediately following high-pressure treatment at 600Mpa for 15minutes at 25°C. Corn starch (——) and Wheat Starch (------).

Crystallization of HHP treated starch during storage

HHP treated corn and wheat starches were stored at 4°C and 23°C. Starch samples were removed periodically and DSC thermograms were recorded. Figure 5 shows the melting endotherm of starch crystals for corn and wheat starches after a storage time of 31 days at 4°C. The thermograms show that the crystallization characteristics of corn and wheat starches are different. The enthalpies, the onset, and peak temperatures of HHP treated corn and wheat starches were 0.96 Jg^{-1} and 0.74 Jg^{-1}, 42.3 °C and 39.3°C, and 53.6°C and 50.5°C.

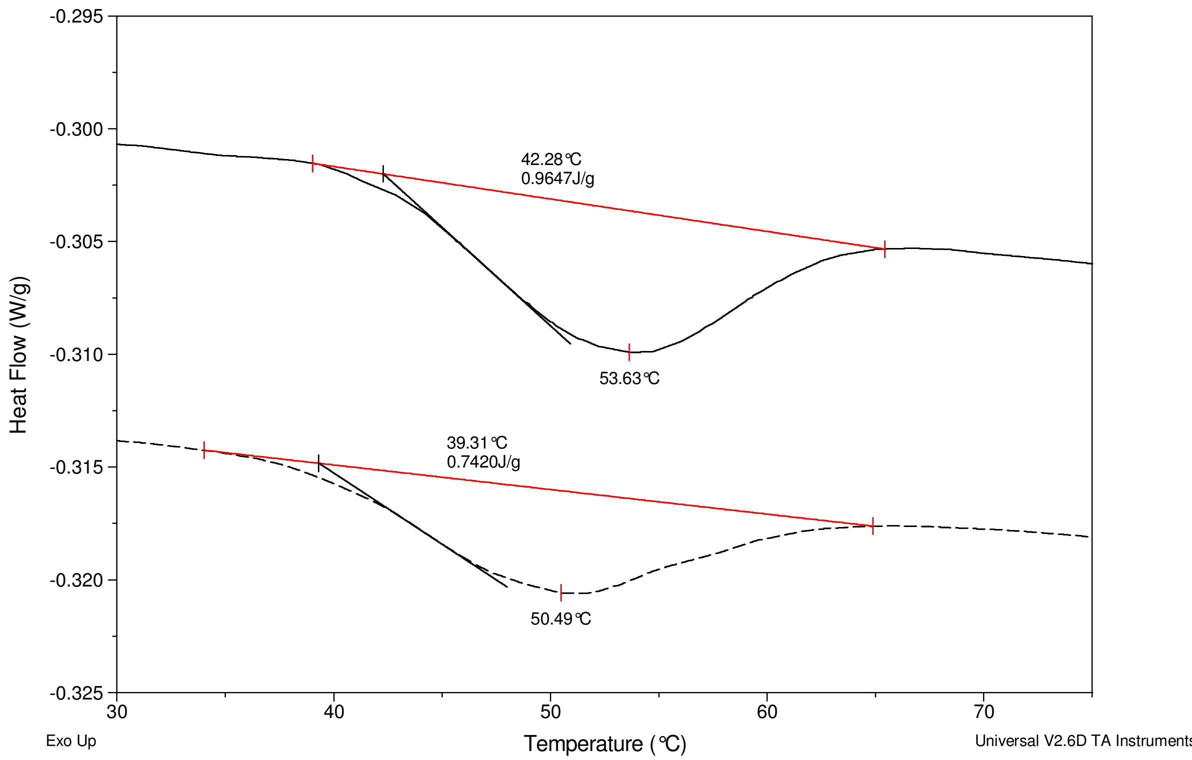

Figure 5: Endotherms of pressure treated corn and wheat starch after 31 days of storage at 4˚C. Corn Starch (——) and Wheat Starch (------).

The enthalpy data were fitted to the Avrami equation to evaluate the crystallization kinetic parameters of HHP treated starch. Figures 6 and 7 show the experimental data and the fitted curve for corn and wheat starch at 4°C and 23°C.

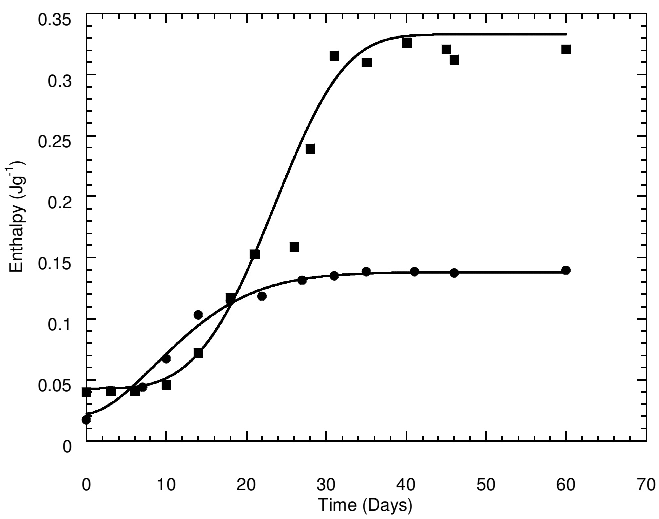

Figure 6: Recrystallization of pressure treated corn and wheat starch stored at 23°C as a function of time. Corn starch (■) and wheat starch (●).

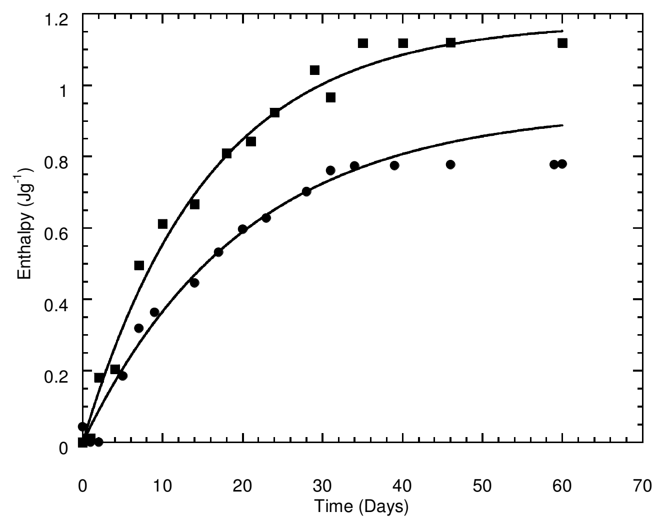

Figure 7: Recrystallization of pressure treated corn and wheat starch stored at 4°C as a function of time. Corn starch (■) and wheat starch (●).

The parameters for the Avrami equation and the asymptotic value of melting enthalpy of starch crystals are summarized in Table 1. Comparison of temperature dependence of crystallization show that both the fractional crystallinity (α) and the rate constant (k) of crystallization increases as the temperature decreases for both starches. The asymptotic heat energy required for melting of starch crystals formed during storage was 0.32 J/g and 0.14 J/g, for corn and wheat starch stored at 23°C and was 1.17 J/g and 0.79 J/g for corn and wheat starch stored at 4°C. The Avrami exponents (n) were found to be different for the corn and wheat starch stored at 23°C (2.5 for corn starch and 1.7 for wheat starch) and stored at 4°C (1.7 for corn starch and 1.2 for wheat starch).

Starch	Temperature (°C)	ΔH_f (J/g)	ΔH_i (J/g)	k $(1/\text{Days})^n$	k' (1/Days)	n	Half Time (Days)
Corn	23	0.32	0.03	0.0002	0.0400	2.7	21.7
	4	1.10	0.10	0.0140	0.0770	1.7	10.5
Wheat	23	0.14	0.02	0.0100	0.0765	1.7	12
	4	0.79	0.10	0.0380	0.0760	1.2	11.1

Table 1: Avrami equation parameters for corn and wheat starch.

Rheological properties of HHP treated starch during storage

A stress sweep (Figure 8a) at 1 Hz frequency was performed to determine the linear viscoelastic region for all HHP processed starch samples at various times during storage. Then, a frequency sweep (Figure 8b) was performed at a constant stress value determined based on the stress sweep test. Typically frequency sweep test were conducted at 1 Pa stress with a corresponding value of approximately 1% strain level.

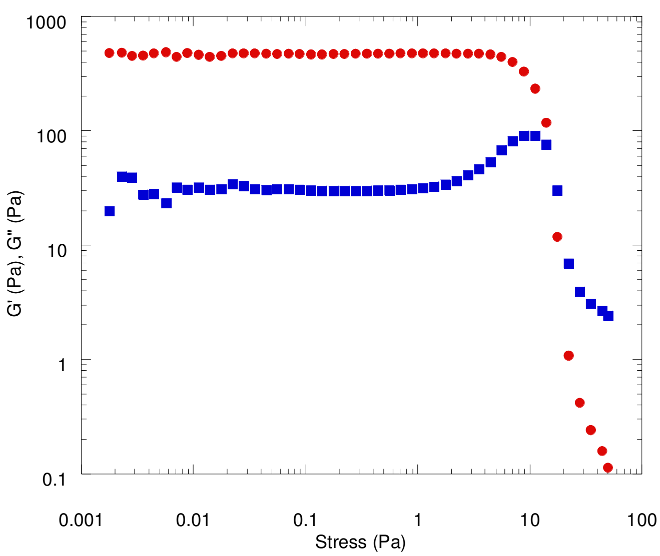

Figure 8a: Stress sweep of corn starch immediately after HHP processing. G' (●), G" (■).

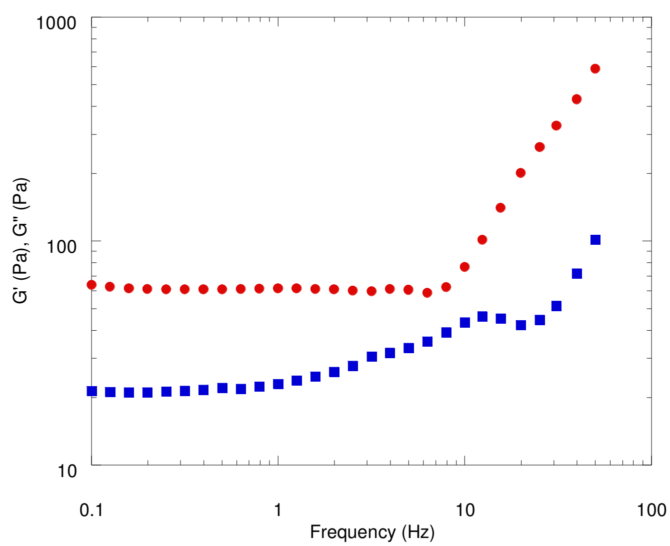

Figure 8b: Frequency sweep of corn starch immediately after HHP processing. G' (●), G" (■).

The G' or G" data above which the linear elastic conditions in a frequency sweep test were not maintained, such as above 10 Hz frequency in Figure 8 b, were not included in analysis of data.

Loss modulus for HHP treated corn starch stored at 23°C appear to be comparatively stable up to 31 days followed by a steep reduction (Figure 9a), while the loss modulus for HHP treated wheat starch stored at 23°C gradually decreased as a function of time (Figure 9b).

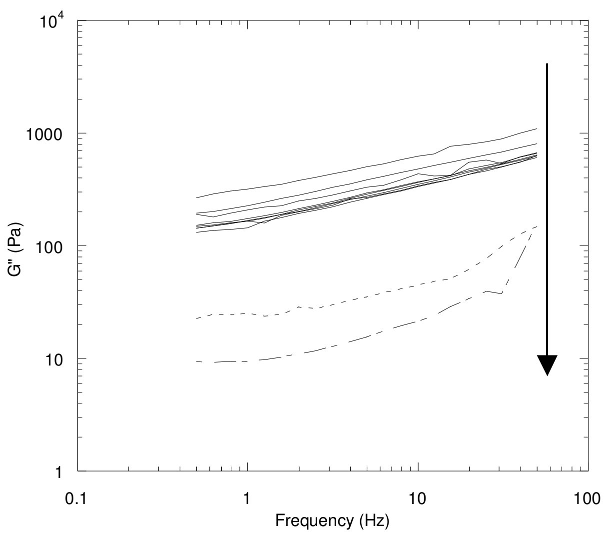

Figure 9a: Corn starch stored at 23°C. Loss Modulus (G") as a function of frequency over time. Storage time increases in direction of the arrow. Solid lines through day 31, Day 34 (---), Day 38 (-).

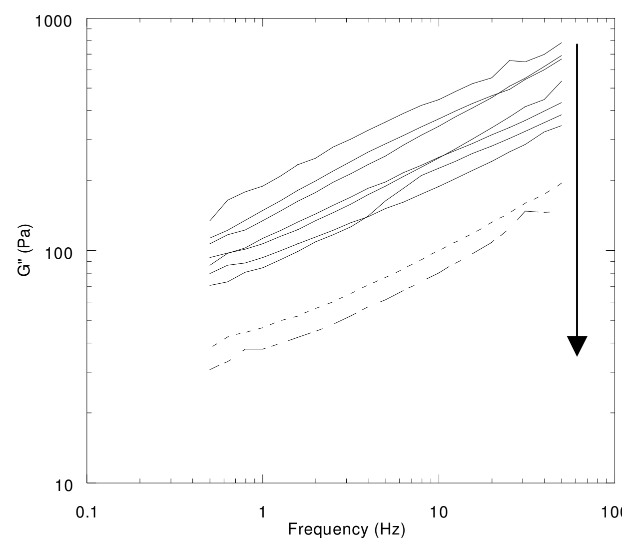

Figure 9b: Wheat starch stored at 23°C. Loss Modulus (G") as a function of frequency over time. Storage time increases in direction of the arrow. Solid lines through day 28, Day 31 (---), Day 35 (-).

HHP treated corn and wheat starches were less stable at low storage temperature of 4°C in comparison with storage at 23°C. Loss modulus decreased 10 fold in 24 hours following HHP treatment for corn starch (figure 10a), while a 20% decrease was observed for wheat starch after 7 days of storage (figure 10b).

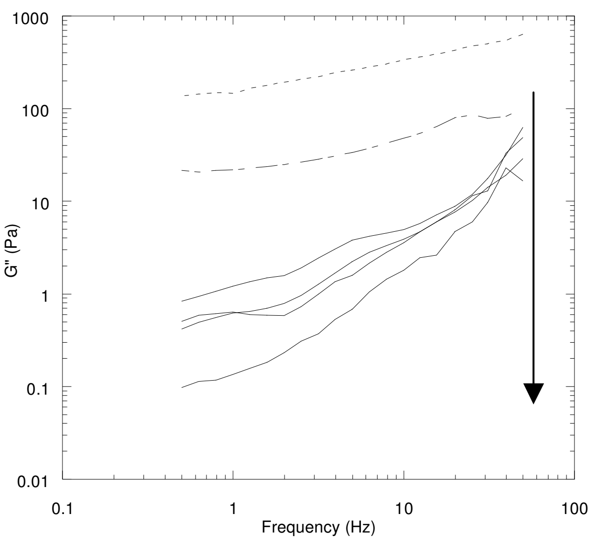

Figure 10a: Corn starch stored at 4°C. Loss Modulus (G") as a function of frequency over time. Time increases in direction of the arrow. Day 0 (---), Day 2 (— - —), solid lines are through 14 days of storage.

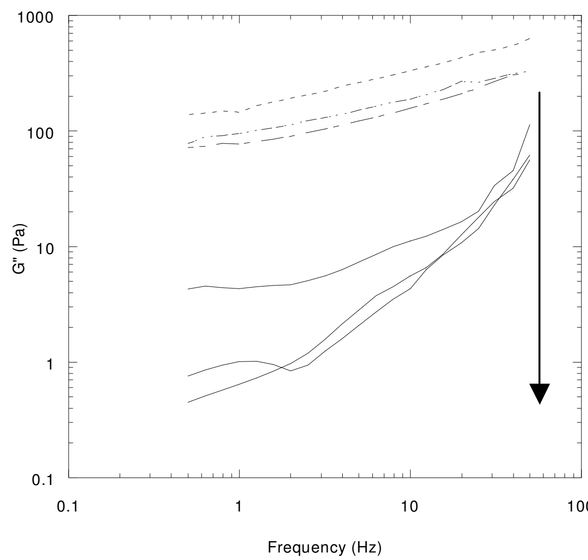

Figure 10b: Wheat starch stored at 4°C. Loss Modulus (G") as a function of frequency over time. Time increases in direction of the arrow. Day0 (---), day2 (— - —), day3 (-- — --), solid lines are through 14 days of storage.

The slope of loss modulus versus frequency (dynamic viscosity, η') was calculated and plotted against storage time for HHP treated corn and what starch (figures 11a and 11b). Corn and wheat starch stored at 23°C displayed a constant dynamic viscosity for 27 days, followed by an increase for corn starch, while it decreased after 30 days for the wheat starch. For both starches stored at 4°C, η' was constant for the first 2 days, followed by a rapid increase.

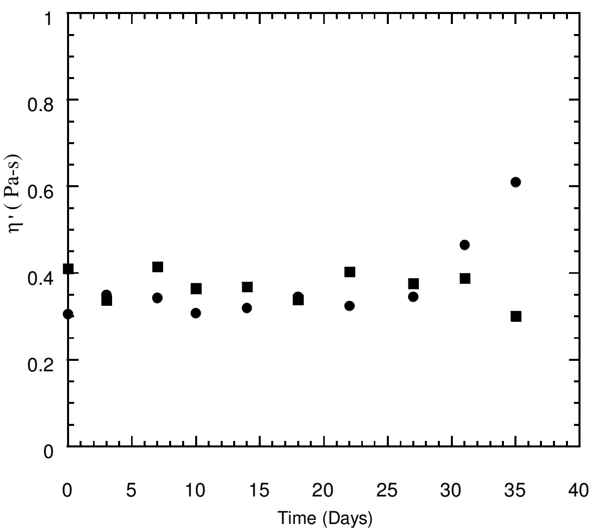

Figure 11a: η' as a function of time for corn (■) and wheat (●) starch stored at 23°C.

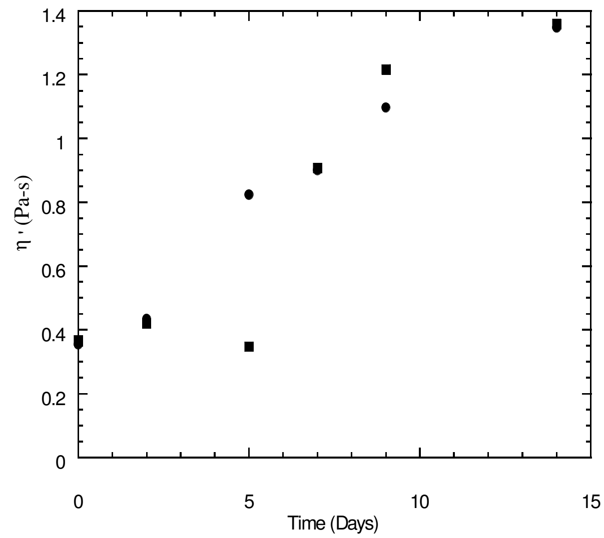

Figure 11b: η' as a function of time for corn (■) and wheat (●) starch stored at 4°C.

Storage modulus for HHP treated corn and wheat starch was calculated at 1 Hz. The data were plotted as a function of time at 23°C and 4°C (Figure 12). G' for HHP treated corn and wheat starch stored at 23°C were stable until day 26 and 40 respectively. However, G' for the HHP treated corn and wheat starch stored at 4°C were highly unstable and decreased after 1 day and 3 days of storage respectively.

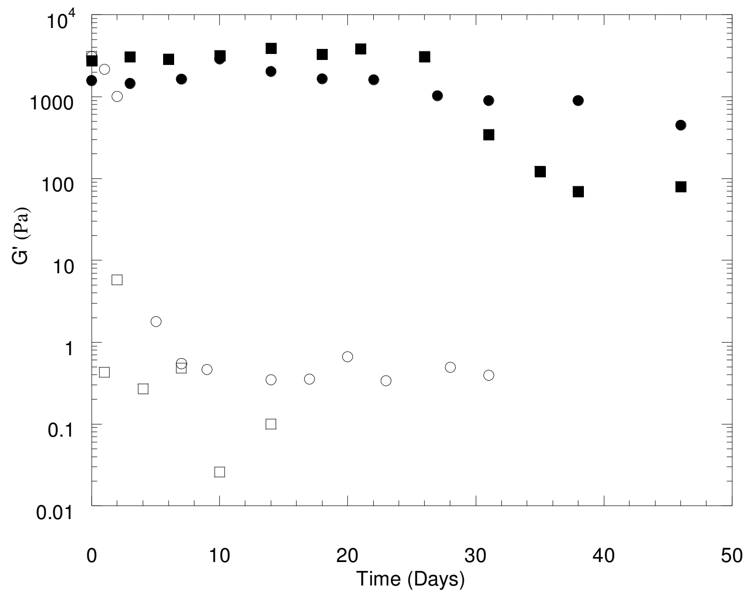

Figure 12: Storage Modulus (G') as a function of frequency at 1Hz over time. Corn (■) and wheat (●) starch stored at 23°C. Corn (□) and wheat (❍) stored at 4°C.

Values of G' and G" directly after HHP processing were 2624 and 144 for corn starch and 1649 and 107 for wheat starch respectively. A comparison of the G' and G" values at 1 Hz after 14 days of storage show that the values for corn starch stored at 23°C were 5020 Pa and 319 Pa respectively. Similarly, for wheat starch stored at 23°C after 14 days, the G' and G" were 2343 Pa and 149 Pa. On the other hand, G" at 1 Hz for corn and wheat starch stored at 4°C for 14 days were 0.14 and 1.01 respectively which were larger than G' values (0.09 for corn starch and 0.49 for wheat starch).

Starch	$\tan\delta = \frac{G''}{G'}$		
	Day 0	Day 14 23°C	Day 14 4°C
Corn	0.06	0.06	1.50
Wheat	0.06	0.06	2.06

Table 2: G"/G' calculated on days 0 and 14 after storage.

Effect of HHP processing on starch suspension stability

HHP or thermally processed and freeze dried corn and wheat starch were used to prepare starch suspensions at 5% starch (w/w) concentration. Figure 13 shows the starch suspension immediately after preparation (cuvette on the left) and 24 hours after preparation (cuvette on the right) for native corn starch.

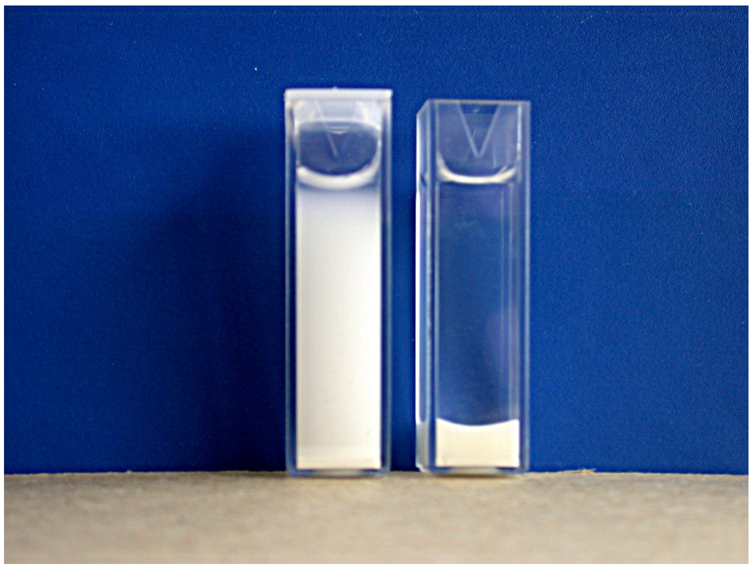

Figure 13: 5% corn starch suspensions before and after settling.

Two types of analysis were used to evaluate the effect of starch botanical origin and processing on suspension stability. The time dependence of starch settling properties were quantified by monitoring transmittance of visible light through the suspension as a function of time.

Figures 14 a, b, c, and d show the percent transmittance for native, HHP treated and thermally processed wheat or corn starch suspensions as a function of time. The time dependence of settling behavior appears to depend on both botanical origin of starch and processing applied. However, all transmittance versus time data appear to have four characteristic segments including: a decrease in transmittance, a lag time, a steep increase in transmittance, and an asymptotic transmittance value. Initial segment of a typical transmittance-time curve shows a decrease in transmittance for the first 20 to 30 minutes of the curve (figure 15). A lag time defined as the time from the start of the test to the beginning of the steep increase of the curve is shown in figure 16. It appears that both the lag time and the initial rate of the settling depend on the botanical origin of starch.

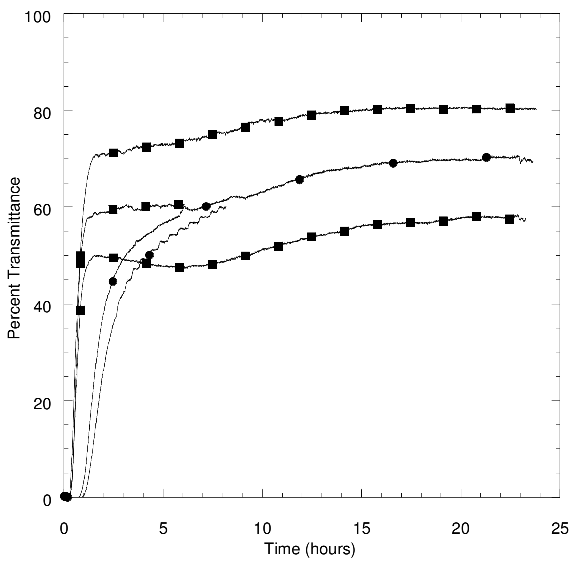

Figure 14a: Transmittance of native corn (■) and wheat (●) starch as a function of time.

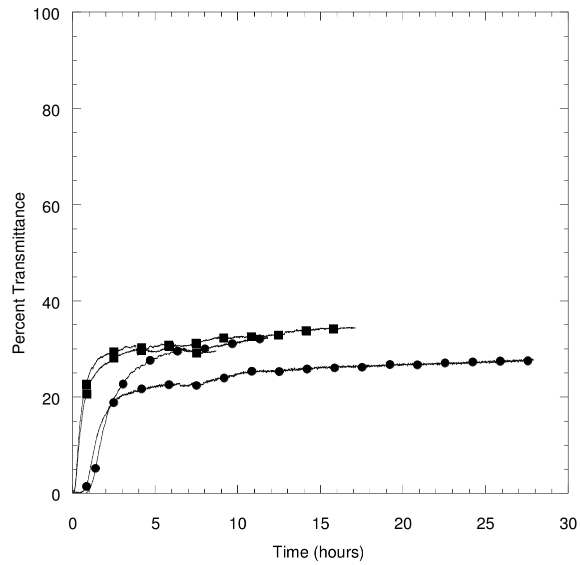

Figure 14b: Transmittance of HHP processed corn (■) and wheat (●) starch as a function of time.

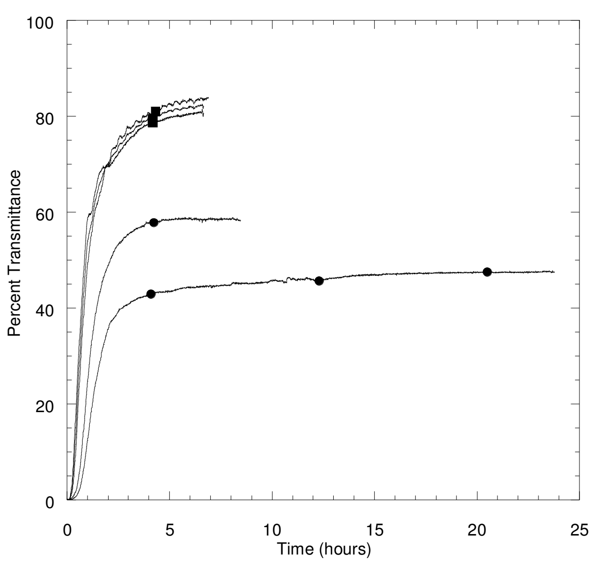

Figure 14c: Transmittance of pre-HHP processed pH 9 corn (■) and wheat (●) starch as a function of time.

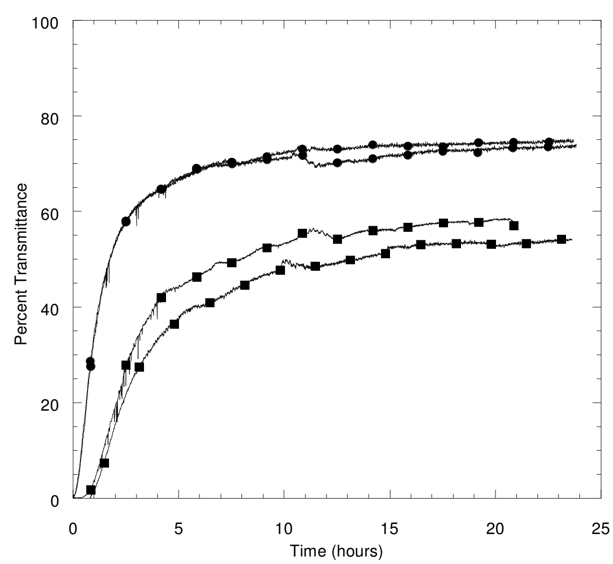

Figure 14d: Transmittance of thermal processed corn (■) and wheat (●) starch as a function of time.

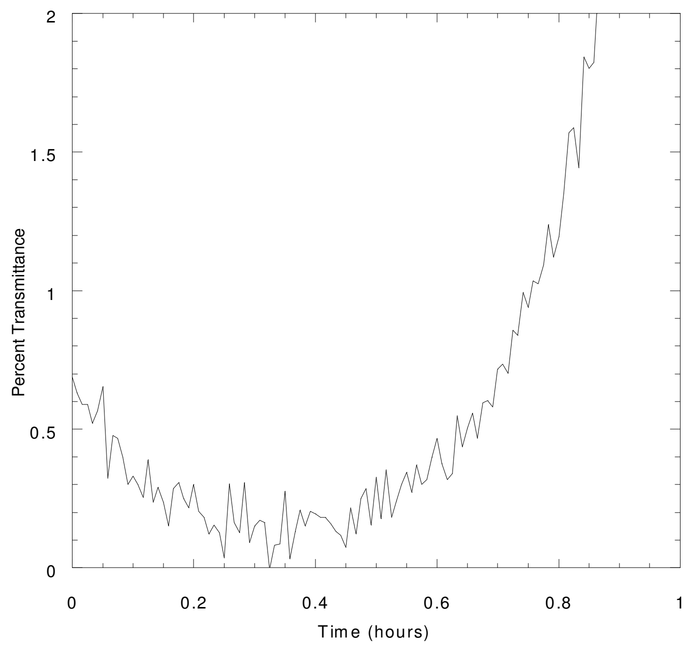

Figure 15: First part of transmittance test for HHP wheat starch.

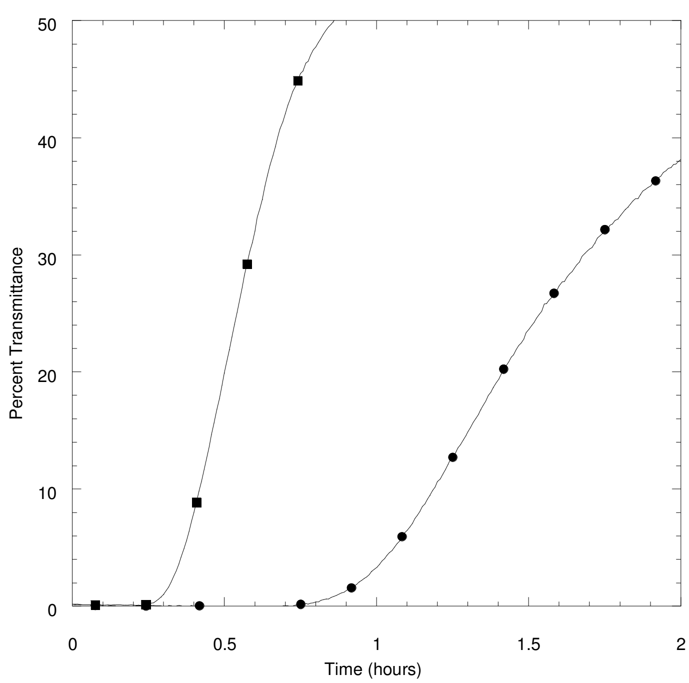

Figure 16: Second and third part of transmittance test native corn (■) and wheat (●).

At the last segment of the settling curve, transmittance data reach to an asymptotic value. Both the asymptotic value of transmittance and the time to reach the asymptotic value depend on the starch botanical origin and processing. The comparison of figures 14 a, b, c, d and 17 shows that HHP processing reduces the extent of settling in aqueous suspensions of starch. The results of transmittance data analysis for settling lag time, initial settling rate, asymptotic value of transmittance and time to reach asymptotic transmittance are outlined in Table 3.

Starch	Lag time (min)	Initial Settling Rate	Asymptotic Transmittance	Time for Asymptotic Transmittance (hours)
NCS	11	122	70	9.5
NWS	48	38	65	14
HCS	36	49	30	9
HWS	63	17	32	11.5
TCS	30	13	56	20
TWS	3	47	74	22
HCS9	6	74	83	6.8
HWS9	9	37	53	11.5

Table 3: Lag time, slope, asymptotic value, and the time to reach the asymptotic value for native HHP and thermal treated corn and wheat starch. Native corn and wheat starch NCS and WCS, HHP processed corn and wheat starch HCS and HWS, thermally processed corn and wheat starch TCS and TWS, HHP processed corn and wheat starch HCS9 and HWS9.

Separation of corn and wheat starch suspensions

The ratios of liquid suspension and settled solid height to total height of native, HHP treated, or heat treated corn and wheat starch are shown in figure 17. The native corn and wheat starch show a similar amount of solid separation (8%) that are different from HHP processed modified starches. HHP processed corn and wheat starch show similar solid separation, both being less than corresponding native starches. The least solid separation was observed for HHP processed pH 9 wheat starch.

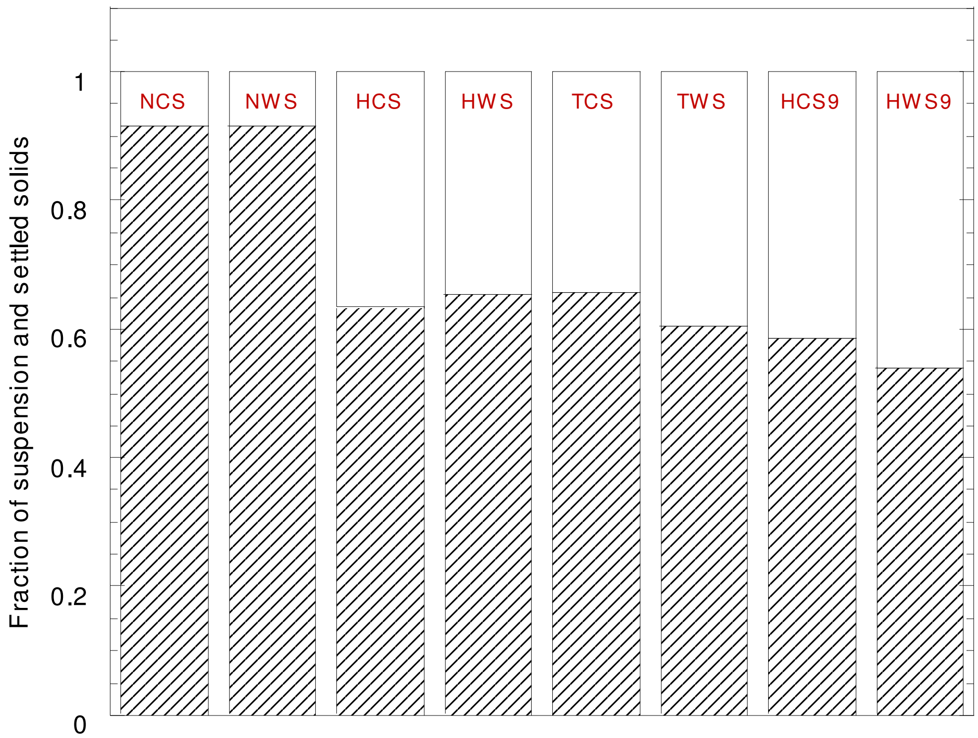

Figure 17: Separation of Native, control, and pre-pH 9 corn and wheat starch suspensions after 24 hours (settled solid , suspension). Native corn and wheat starch NCS and WCS, HHP processed corn and wheat starch HCS and HWS, thermally processed corn and wheat starch TCS and TWS, HHP processed corn and wheat starch HCS9 and HWS9.

Discussion

This project investigated the effect of HHP processing on starches from different botanical origin. Specifically, crystallization characteristics of HHP treated starch gels and settling properties of aqueous suspensions of starches after HHP treatment were studied and compared to those of native and thermally processed starches.

Starches from different botanical origins were known to have different structural and functional properties. As reported in literature (Roos, 1995), our experiments show that thermally induced gelatinization required higher temperatures and enthalpy for corn starch than wheat starch suggesting a more thermally stable crystalline structure for corn starch.

The HHP processing conditions for complete gelatinization of starch were found to be in agreement with the data reported in literature. Based on some of the results reported in literature (Douzals et al, 1996; Douzals et al, 1998) and the results obtained from this study, both corn and wheat starch are completely gelatinized by pressure at 600 MPa and at 25˚C over the starch concentration range (5-30% w/w) tested. However, Zuo et al (1999) reports the presence of native starch after treatment at 700 MPa for 2min but complete gelatinization after 5 min for corn starch. The results suggest that starch can be partially or completely gelatinized by changing pressure at constant temperature and time as well as by changing time at constant pressure and temperature.

Corn and wheat starch crystallized during storage at both 23°C and 4°C. However, the rate and extent of crystallization depend both on starch botanical origin and the storage temperature. Effect of storage temperature on the overall crystallization rate, which is influenced by both nucleation and crystal growth rates, is well established.

Levine and Slade (1991) state that starch gels that are stored between the glass transition and melting temperatures exhibit crystallization and the overall rate of crystallization decreases as the storage temperature approaches either to the glass transition or to melting temperature. Maximum rate of crystallization is to be expected at a temperature corresponding to the midpoint in a temperature range between glass transition and melting temperatures. The glass transition temperature for starch-water system (15% w/w) used in the present study was calculated to be –126.5°C using the Couchman-Karasz equation (Couchman and Karasz, 1978). The melting temperature of crystals for the present study measured by using DSC was 70°C for corn starch and 64°C for wheat starch. Therefore, the maximum rate of crystallization for corn and wheat starch was expected to be at approximately –28°C and –31°C respectively. Because the temperatures expected to result in maximum amount of crystallization for both starches are closer to 4°C, the higher amount of crystallization observed for starches stored at 4°C in comparison with starches stored at 23°C is in agreement with literature.

The Avrami equation parameters corresponding to crystallization rate constant (k) and the exponent describing nucleation and growth mechanisms (n) depend on the botanical origin and storage temperature. A value of one suggests instantaneous nucleation and rod-like growth, which can be used to describe the crystallization behavior of wheat starch at 4°C. A value of 2, is either considered as two dimensional growth with instantaneous nucleation or rod-like growth with a heterogeneous random nucleation. The crystallization of wheat at 23°C or of corn at 4°C exhibit 1.7, a value close to two. Avrami exponent originally assumed to be only an integer value. A fractional value such as 1.7 was obtained in other studies and attributed to a complex crystallization of one and two dimensional growth with an instant nucleation. The shapes of the curves in figures 7 and 8 indicate that crystallization of starch starts immediately following processing for all conditions tested except for corn starch stored at 23 °C. Previous studies on crystallization of rice starch (Moo-Yeol et al, 1997) and of potato starch (Jagannath et al, 2001) report a value of 1 for Avrami exponent following a thermal treatment. Both the presence of a lag time in starch crystallization curve and an *n* value of 2.7 for corn starch stored at 23 °C may be attributed to a random nucleation followed by a two dimensional growth. In addition to the rate of crystallization which is expected to be affected by storage temperature, the extent of crystallization was different for starches stored at 4 and 23 °C although the initial starch concentrations were identical. At both storage temperature wheat starch exhibited less crystallization compared to corn starch. The recrystallization of the starch was attributed to amylose within the starch granules (Douzal et al, 1998; Rao, 1999). However, Fennema (1996), states that the crystallization of starch happens in two stages, the first is attributed to amylose and the second is due to the amylopectin. Because the amylose/amylopectin contents are similar for both starches, the difference in extent of crystallization can not be attributed to amylose or amylopectin content.

Storage modulus, G’, is a parameter describing the strength of a gel. A strong gel is considered to have a G’ value of at least two fold greater than G” value. A gel could definitely be considered as a strong gel if G’ is at least 10 times greater than G” throughout the frequency range studied in the experiment (Clark and Ross-Murphy, 1987, Rao and Steffe, 1992). The magnitudes of G’ and G” for corn and wheat starch show similar results for G’ (2500 Pa) and G” (200 Pa) immediately following pressure treatment implying a strong gel formation following pressure treatment at 600MPa for 15 minutes at 25°C. G' at 1 Hz as a function of time (figure 11) suggests that the corn starch and wheat starch maintained strong gel characteristics for 28 and 32 days respectively. A comparison of tan δ values for samples immediately after processing and after 14 days of storage also shows that while the gels stored at 23 °C show tan δ values in the order of magnitude typical for gels, the gels stored at 4 °C exhibit values similar to concentrated solution (Steffe, 1996). The analysis of dynamic viscosity, η', values also confirm the results as they increase for gels stored at 4 °C about two after processing while the increase of η' does not occur for about 30 days for HHP treated starches stored at 23 °C.

A comparison of the rheological and thermal properties of HHP treated starch provide a valuable tool to characterize the crystallization phenomena during storage. The DSC results show that during storage the amount of crystallization in starch increases, while the rheological data shows that during storage η' and tan δ increase. However, the increase in rheological properties is not observed until a critical value of starch crystallization is reached.

HHP processing appears to affect both the settled solid separation and the turbidity of the starch suspension. Native corn and wheat starches (figure 14a) settle at a final transmittance value that is two times greater than that of the HHP processed modified control corn and wheat starch (figure 14b), confirming that HHP processing modified the settling properties of both corn and wheat starch. The differences between the settling characteristics of corn and wheat starches could be due to the differences in particle size and/or due to the differences in the swelling characteristics upon suspension in water. Heganbart (1996) states that corn and wheat starch contain 25% amylose, but the shape and size of the granules are different. Corn starch has irregular polyhedron-shaped granules that range in sizes between 5 to 20 microns, while wheat starch has both larger, 5 to 15 microns, and smaller, 2 to 3 microns, granules (Heganbart 1996). The separation data collected confirms that HHP modification of starch has an affect on both the corn and wheat starch (figure 17) suggesting use of HHP modified starch could be advantageous in the food industry to increase the shelf life of suspended liquid products.

While the study of phase separation gives a quantitative measure of solid and liquid phases separated, spectroscopic study provides the change in suspension concentration in liquid phase as a function of time. Figure 17 shows that a smaller amount of settled solid height for native starch in comparison with HHP treated starch suggesting less solid separation in native starch. However, a close examination of figure 14 a and b shows that a lower transmittance for HHP treated starch than that of native starch indicating more suspended solids in HHP treated starch suspension. Therefore, the information from the two analyses is complementary to each other and is necessary for a comprehensive characterization of suspensions.

Turbidity measurements depend on the concentration of the suspended particles in the volume through which transmittance measurements are collected. During lag time, in all samples a decrease in transmittance was observed. The decrease is attributed to concentration of settling solids in the sampled volume at the level of light source. The time window during which a decrease of transmittance is recorded can be changed by changing the level of the light source going through the cuvette.

A sharp increase in transmittance is observed as the interface between the settled material and the lower concentration suspension in the upper plane passes through the sampled volume. The subsequent slower increase in transmittance is due to settling in the lower concentration upper phase.

Both thermal processing and HHP processing modify the starch so that settling of solids in suspension is reduced similarly according to phase separation. However, it is interesting to note that the while the asymptotic transmittance are similar for both HHP treated starches, thermally processed wheat starch showed a higher solid concentration in suspension than did corn starch. These results suggest that HHP and thermally processing affect corn and wheat starch differently.

Conclusion

The results of the DSC study showed that wheat starch stored at 23˚C over time showed the least amount of crystallization and the least structural loss; therefore it exhibited the highest physical stability. Results of both the DSC and rheological properties study show that the corn and wheat starch stored at 23°C was stable for a longer period of time, compared to starch stored at 4C. DSC and rheometer results also show that retrogradation of HHP processed corn and wheat starch gels depend on the storage temperature and botanical origin of the starch.

The settling characteristics of the starch were studied by turbidity and settled solid and suspension measurements. Transmittance data that were collected for the turbidity measurements show that HHP processed corn and wheat starch show the smallest asymptotic transmittance value. Measurements of the settled solid and suspension over a 24 hour period show that thermal treatment and HHP processing affect native corn and wheat starches settling characteristics. Furthermore, the settling characteristics also were dependent upon the botanical origin of the starch and processing conditions.

This study shows that HHP processing affects the physical properties of starch differently than thermal processing. The properties of the starch are also dependent upon storage time, and temperature, processing conditions, and the botanical origin of starch. Overall HHP modification of native corn and wheat shows potential to be used in commercially processed foods.

Recommendations and Suggestions

The experiments in this study demonstrated that HHP processing modify the starch particles resulting in increased suspension stability. The increased stability could be due to swelling of starch particles. Therefore a thorough study should also include the effect of HHP processing on particle size distribution of starch and furthermore the effect of particle size distribution on settling properties of starch. During HHP processing starch particles swell. However, if the starch granules do not swell uniformly during high pressure processing another variable will be introduced into the study, which will skew the results. A recommendation to solve these problems would be to sieve the starch particles to a uniform size and study the effect of particle size on the settling characteristics.

A cuvette holder should be built to study the effect of temperature on the settling properties relevant to storage conditions of foods. Figure 18 is a design that I believe can be used to provide a temperature control. This design also provides a means to monitor the starch suspension at 3 levels inside the container. It has an insulated chamber for the cuvette, the temperature will be controlled by an external water bath by circulating constant temperature water during the experiment.

During crystallization of starch in gels, synerisis is observed. Because it is expected that syneresis to affect crystallization as well as the rheological properties, I recommend that it should be considered in an experimental design.

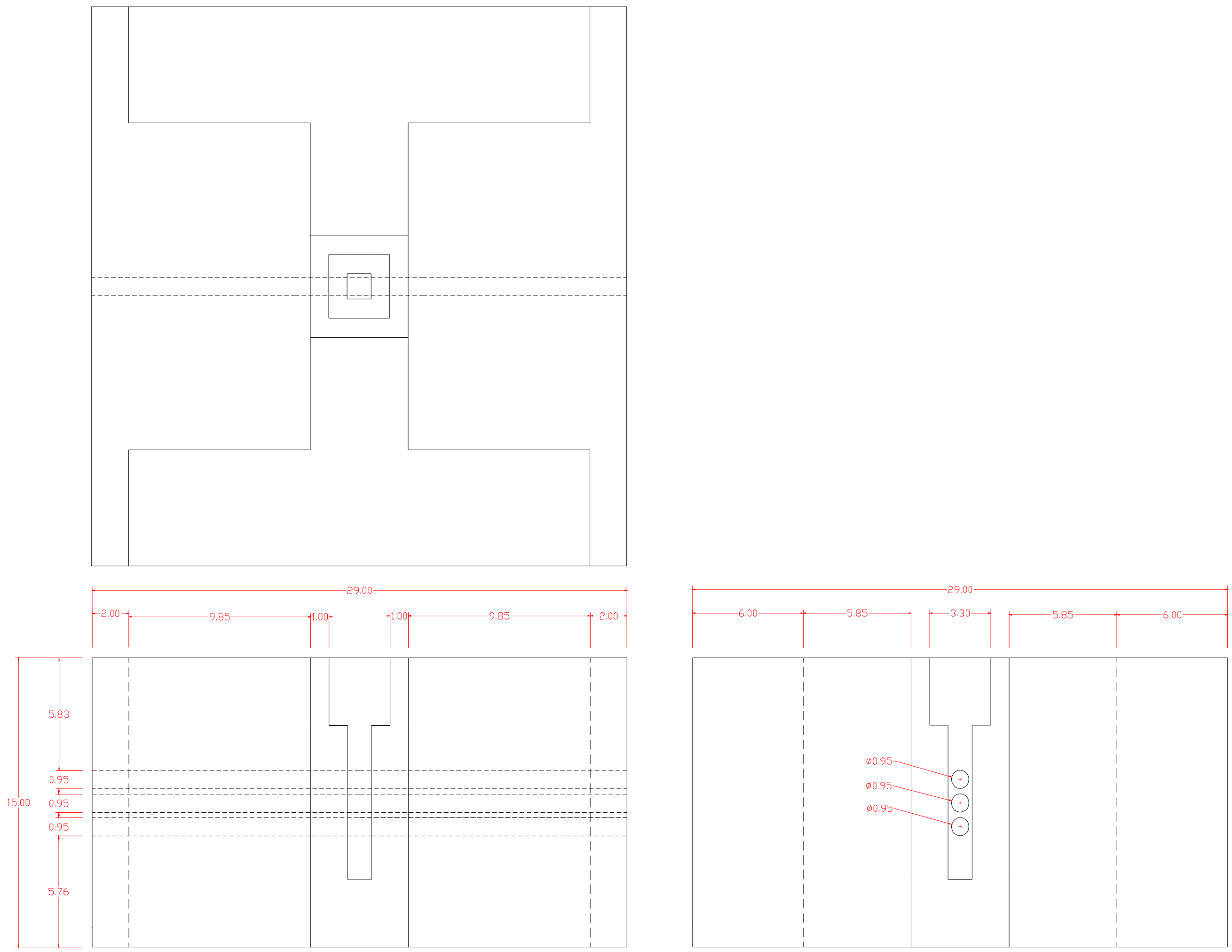

Figure 18: Top, front, and side view of spectrophotometer cell design. All dimensions are in centimeters.

Literature Cited

Avrami, M. 1939. Kinetics of phase change. I. General Theory. J. Chem. Phys.

Clark, A. H., & Ross-Murphy, S. B. 1987. Structural and mechanical properties of biopolymer gels. Advances in Polymer Science, 83, 55-192.

Couchman, P. R. & Karasz, F. E. 1978. Macromolecules. Viscoelastic Properties of Polymers 2^{nd} edition, 11, 117-119.

Douzals, J.P., Marechal, P.A., Coquille, J.C. & Gervais, P. 1996. Microscopic study of starch gelatinization under high hydrostatic pressure. Journal of Agricultural and Food Chemistry, 44, 1403-1408.

Douzals, J.P., Cornet, J.M. Perrier, Gervais, P. & Coqulle, J.C. 1998. High-pressure gelatinization of wheat starch and properties of pressure-induced gels. *Journal of Agricultural and Food Chemistry,* 46, 4824-4829.

Famelart, M.H., Chapron, L., Piot, M., Brule, & Durier C. 1998. High pressure-induced gel formation of milk and whey concentrates. *Journal of Food Engineering*, 36, 149-164.

Farkas, F., Daniel & Hoover, G., Dallas 2000. High pressure processing. *Journal of Food Science*, 47-64.

Fennema, Owen R. 1996. *Food Chemistry Third Edition*. Pages 195-198, 201-204. Marcel Dekker, Inc: NY, NY.

Heganbart, Scott 1996. Food Product Design: Understanding Starch Functionality. Weeks Publishing Co: Northbrook, IL. http://www.foodproductdesign.com/archive/1996/0196CS.html.

Jagannath, J.H., Nanjappa, C., Das Gupta, D.K., & Arya, S.S. 2001. Crystallization kinetics of precooked potato starch under different drying conditions (methods). *Food Chemistry*, 75, 281-286.

Jobling, Steve 2004. Improving starch for food and industrial application. *Current Opinion in Plant Biology*, 7, 210-218.

Jouppila, K. and Roos, Y.H. 1997. The physical state of amorphous corn starch and its impact on crystallization. *Carbohydrate Polymers*, 32, 95-104.

Jouppila, K., Kansikas, J., Roos, Y.H. 1998. Factors affecting crystallization and crystallization kinetics in amorphous corn starch. *Carbohydrate Polymers*, 36, 143-149.

Levine, Harry and Slade, Louise 1991. *Water Relationships in Foods*. Plenum Press, New York, New York.

Li, Jian, Zhou, Chixing, Wang, Gang, Tao, Ying, Lui, Auing, Li, Yang 2002. Isothermal and nonisothermal crystallization kinetics elastomeric polypropylene. *Polymer Testing*, 21, 583-589.

Messens, W., Van Camp, J. & Huyghebaret, A. 1997. The use of high pressure to modify the functionality of food proteins. *Trends in Food Science and Technology*, 8, 107-112.

Moo-Yeol Baik, Kwang-Joong Kim, Ki-Cheol Cheon, Yeon-Chul Ha, and Wang-Soo Kim, 1997. Recrystallization kinetics and glass transition of rice starch gel system. *Journal of Agricultural Food Chemistry*, 45, 4242-4248.

Polydera, A.C., Stoforos, N.G., & Taoukis, P.S. 2003. Comparative shelf life study and vitamin C loss kinetics in pasteurized and high pressure processed reconstituted orange juice. *Journal of Food Engineering*, 60, 21-29.

Rao, Anandha M. 1999. *Rheology of Fluid and Semisolid Foods*. Pages 204-206. Aspen Publishers: Gaithersburg, Maryland.

Rao, Anandha M. & Steffe J. F. 1992. *Viscoelastic Properties of Foods*. Elsevier Dcience Publishers LTD: England.

Roos, Y.H 1995. *Phase Transitions in Food*. Academic Press: San Diego, California.

Rubens, P. & Heremans, K. 2000. Pressure-temperature gelatinization phase diagram of starch: an in situ fourier transformation infrared study. *Biopolymers*, 54, 524-530.

Steffe, James 1996. *Rheological Methods in Food Process Engineering*. Pages 348-349. Freeman Press: East Lansing, MI.

Stolt, M., Oinonen, S. & Autio K. 2000. Effect of High Pressure on the Physical Properties of Barley Starch. *Innovative Food Science & Emerging Technologies*, 1,167-175

Stute, R., Heilbronn, Klingler, R.W., Boguslawski, S., Eshtiaghi, M.N., & Knorr, D. 1996. Effects of High Pressures Treatment on Starches. *Starch/Starke*, 48, 399-408.

Walter, H. Reginald 1998. Polysaccharide Association Structures in Food. Pages 57-59. Marcel Dekker, Inc: NY, NY.

Zuo Chungcheng, Ma, Chenglin, Zhang Shouqin 1999. Effects of High Hydrostatic on Gelatinization of Corn Starch. Pages 91-93 in: Volume 4 proceedings of the International Conference On Agricultural Engineering (ICAE), Beijing, China 1999.